

# Exhibit G

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Research

## Differences in polypropylene shrinkage depending on mesh position in an experimental study

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### Abstract

**Background:** Polypropylene (PP) mesh is one of the most frequent materials used in hernia repair. We have experimentally evaluated the shrinkage of PP mesh depending on the place of implantation.

**Methods:** In 15 New Zealand rabbits a muscular defect measuring 3 × 3 cm was created in both pararectal sides of the abdominal wall. The defect was repaired using a PP mesh measuring 5 × 3.5 cm that was placed in the right side in the sublay location and in the left side in the onlay location. Five animals were killed on the 30th, 60th, and 90th postoperative days. Macroscopic measurement and microscopic study of the prosthesis–host tissue interfaces were performed.

**Results:** One rabbit was killed because of severe infection in the onlay mesh. Another 2 infections were tolerated in the onlay mesh side. All the prostheses were integrated in the host tissue at death. In the macroscopic evaluation the mesh areas were reduced by 25.92% on the 30th day, by 28.67% on the 60th day, and by 29.02% on the 90th day. The mesh shrinkage was greater in the onlay group than in the sublay group at the 3 time intervals. More inflammatory leukocyte and mononuclear responses also were seen in the onlay group.

**Conclusions:** These observations support the theory of PP mesh shrinkage as a consequence of the incorporation of the biomaterial to the scarring tissue. This shrinkage is significantly more intense if the meshes are placed in the onlay position. © 2007 Excerpta Medica Inc. All rights reserved.

**Keywords:** Hernia; Surgical mesh; Polypropylene; Shrinking

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Polypropylene (PP) mesh is universally accepted for use in the repair of incisional hernias [1]. This mesh was introduced in 1958 by Usher et al [2], and later was popularized by Lichtenstein [3]. This material has been proven not to be completely inert and does generate an inflammatory response as a foreign body reaction that differs between individuals and depends on the amount of material and the structure of the mesh [4–6]. In fact, late complications such as chronic infection, migration, and erosion have been described.

One of the physical consequences of the inflammatory response to the mesh is shrinking, which has been responsible for recurrences and pain [7,8]. A certain degree of shrinkage,

contraction, or folding of the mesh has been reported in experimental models and in some clinical reports during the past 8 years. We also postulated that shrinkage might be the reason for recurrences in the mesh borders after an onlay mesh ventral hernia repair, as seen in our clinical practice. These recurrences were not observed after sublay repairs. The aim of our study was to confirm this contraction in an experimental model and to evaluate possible differences in shrinking depending on the position of implantation.

### Materials and Methods

The study was approved by the Cadiz University Committee of Experimental Studies. Fifteen female New Zealand rabbits weighing 2000 to 2500 g were used. The animals followed the European Union guidelines for animal studies (CEE 2871-22A9). All animals were housed in in-

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dividual cages with controlled light/dark cycles, constant temperature, and given free access to water.

All animals were given 150 mg/kg cefazolin 1 hour before the surgical procedure. The anesthesia was induced by an intramuscular injection of ketamine hydrochloride (Ketolar; Parke Davis, Barcelona, Spain) 70 mg/kg and atropine .25 to .3 mL. Additional anesthetic doses were administered in some cases if it was necessary during surgery. Postoperative infiltration with bupivacaine also was used to moderate postoperative pain.

A combined bilateral approach was planned to create 2 groups. An identical defect in the pararectal space was created in either side, a 5-cm long  $\times$  3.5-cm wide PP mesh (Trelex, Boston Scientific Corp.) was implanted on the preperitoneal plane (sublay group) in the right side and on the prefascial plane (onlay group) in the left side. Under sterile conditions the animals were shaved and the surgical field was organized with the animal in a supine position. A vertical 5-cm pararectal incision was made in both sides of the anterior abdominal wall. A 3-cm  $\times$  3-cm wide defect, including all muscular layers, was created in either side. On the right side the retromuscular preperitoneal space was dissected, the peritoneum was closed with 4/0 polyglactin 910 (Vicryl; Ethicon, Somerville, NJ) suture, and a mesh (5  $\times$  3.5 cm) was laid in the layer sandwiched between the peritoneum and the muscle, fixed with interrupted PP 4/0 sutures. Then, the anterior fascia of the abdominal wall was closed with a 4/0 PP running suture. On the left side the defect was closed with 4/0 polyglactin 910 suture, including all the layers, and a mesh (5  $\times$  3.5 cm) was placed on the prefascial plane fixed with 4/0 interrupted sutures. Finally, the skin was closed with 4/0 nylon interrupted sutures.

Eight hours after surgery the animals were left to feed and drink ad libitum. Five animals were killed 30, 60, and 90 days after implantation. The complete anterior abdominal wall was removed for macroscopic and microscopic evaluations. The presence of tissue integration, infection, denuded areas in the implants, seromas-hematomas, and adhesion formation was recorded. The mesh was isolated in either side and measured in the 4 borders. Tissue samples were obtained from the prosthesis interfaces. Conventional light microscopy was performed on 5- $\mu$ m slices after fixing in 10% formaldehyde and embedding in paraffin. The specimens were stained in hematoxylin-eosin, Masson's trichrome, orcein, desmin, CD 68, and factor VIII. A morphometric analysis was performed at the interface within 500  $\mu$ m around the mesh. The partial volume and the percentage of cells were calculated.

Statistical analysis was performed with the nonparametric Wilcoxon rank-sum test to compare measurements be-

Table 2  
Macroscopic abnormal findings seen at 30, 60, and 90 days after implantation

| Findings            | Day 30     |             | Day 60     |             | Day 90     |             | Total |
|---------------------|------------|-------------|------------|-------------|------------|-------------|-------|
|                     | Mesh onlay | Mesh sublay | Mesh onlay | Mesh sublay | Mesh onlay | Mesh sublay |       |
| Infection           | 1*         | —           | 2          | —           | —          | —           | 3     |
| Seroma-hematoma     | 1          | —           | —          | —           | —          | —           | 1     |
| Denuded areas       | —          | 1           | 1          | —           | 1          | —           | 3     |
| Abdominal adhesions | —          | —           | —          | —           | —          | —           | —     |

\* Animal was killed on the 6th day and excluded from the study.

tween both groups. In addition, a multiple linear regression study was applied. SPSS 11.5 software (SPSS Inc., Chicago, IL) was used.

## Results

There were no intraoperative complications. One animal developed a severe prosthetic infection of the onlay mesh and was killed on the 6th postoperative day and excluded from the study. No other mesh was removed. The weights and abdominal perimeters of the animals were increased progressively in both groups (Table 1). Two animals killed on the 60th day had mild postoperative wound infections in the onlay side that were treated effectively with local wound cures. These animals had a favorable evolution and were included in the study. The macroscopic abnormal findings on death are shown in Table 2. All the implants were incorporated into the host tissue. Only a few cases showed small denuded areas in the mesh-tissue interface. The detachment of the implant from the abdominal layers was very difficult in both groups, especially when it was situated in the sublay location. In the explanted specimens, we observed folding of the materials in all cases with a perceptible macroscopic appearance of shrinkage (Fig. 1).



Fig. 1. Macroscopic appearance of shrinkage with foldings of an onlay mesh in an animal killed on the 60th day.

Table 1  
Morphologic data of animals

| Day                              | 30             | 60             | 90             |
|----------------------------------|----------------|----------------|----------------|
| Preoperative weight              | 3667.2 (169.8) | 2749.0 (365.3) | 3953 (300.3)   |
| Weight at death                  | 4993.0 (401.3) | 4344.0 (75.5)  | 5186.4 (483.0) |
| Preoperative abdominal perimeter | 38.5 (1.7)     | 34.0 (.76)     | 37.4 (2.3)     |
| Abdominal perimeter at death     | 42.5 (3.1)     | 41.0 (1.8)     | 43.1 (3.7)     |

Data are expressed as mean (SD).

Table 3

Measurements of mesh at 30, 60, and 90 days after implantation

|                                           | Day 30       |              | Day 60       |             | Day 90      |              |
|-------------------------------------------|--------------|--------------|--------------|-------------|-------------|--------------|
|                                           | Onlay        | Sublay       | Onlay        | Sublay      | Onlay       | Sublay       |
| Length (5 cm) <sup>a</sup>                | 4.02 (.35)   | 4.20 (.21)   | 3.77 (.15)   | 4.27 (.06)  | 3.77 (.09)  | 4.45 (.43)   |
| P value                                   | .012         |              | .012         |             | .005        |              |
| Width (3.5 cm) <sup>a</sup>               | 3.12 (.18)   | 3.20 (.29)   | 3.03 (.26)   | 3.15 (.07)  | 3.90 (.08)  | 3.00 (.20)   |
| P value                                   | .018         |              | .012         |             | .005        |              |
| Area (17.5 cm <sup>2</sup> ) <sup>a</sup> | 12.44 (1.58) | 13.48 (1.88) | 11.49 (1.50) | 13.46 (.36) | 10.94 (.31) | 13.40 (1.73) |
| P value                                   | .012         |              | .012         |             | .005        |              |

Data are expressed as mean (SD).

<sup>a</sup>Initial measurement of mesh at implantation.

In Table 3 the 2-dimensional examination showed a significant shortening of the mesh in length and width on the 30th day ( $P = .01$ ,  $P = .01$ , respectively), the 60th day ( $P = .01$ ,  $P = .01$ , respectively), and the 90th day ( $P = .005$ ,  $P = .005$ , respectively). The implant areas were reduced by 25.92% (onlay, 28.89%; sublay, 22.95%) on the 30th day, by 28.67% (onlay, 34.30%; sublay, 23.05%) on the 60th day, and by 29.02% (onlay, 37.45%; sublay, 23.40%) on the 90th day (Fig. 2).

In the multiple-regression linear analysis the onlay group showed a statistically significant additional shrinking than the sublay group: length,  $P = .001$ ,  $R = -.654$  interval of confidence (IC) (−.6 to −.241); width,  $P = .016$ ,  $R = -.468$  IC (−.43 to −.04); and area  $P = .013$ ,  $R = -.440$  IC (−2.5 to −.32).

In the microscopic evaluation, all of the meshes were integrated into the host tissues with dense scar tissue. Numerous fibrous bundles were arranged paralleled to the prosthetic surface with areas of fibrinoid necrosis. A number of myofibroblasts also was found among fibrous tissue in the 3 periods of study, more frequently found in the onlay meshes (Table 4). There was also a great inflammatory response with infiltration of polymorphonuclear leukocytes including foreign body reaction (granulomas and giant cells) outlining both sides of the biomaterials. This inflammatory response was slightly more intense in the onlay meshes, and in both groups this infiltrate decreased from the

first to the third month. In addition, an increased neoangiogenesis colonizing the connective tissue also was observed in the onlay meshes.

### Comments

Mesh repair is the treatment of choice in abdominal incisional hernias [9]. In the open approach, the PP meshes can be placed in 2 locations safely: onlay, in which the mesh is epifascial on the anterior lamina of the rectus sheath after repair of the defect; and sublay, which is a retromuscular placement of the mesh on the posterior lamina of the rectus sheath or on the preperitoneal spaces. The advantages of the onlay technique are the easier dissection of a risk-free plane over the sheath and the security of laying the mesh far away from the abdominal contents. However, this technique is inconvenient because of the need for an extensive subcutaneous dissection and the limitation of the anatomic boundaries that may restrict the appropriate overlap. Postoperative seromas and infections also are fairly common [10]. The sublay technique promoters advocate that this is theoretically the more correct position to deal with the intra-abdominal pressure forces, and holds the prosthesis against the deep surface of the muscles [11–14]. On the other hand, the longer dissection and separation of the mesh from the abdominal contents is certainly more difficult. There is not enough evidence based on clinical trials to determine whether the sublay location is superior to the onlay location [15]. Our study suggests the benefits of sublay meshes because of fewer infections and less degree of shrinkage.

Despite its high biocompatibility, the PP mesh does generate a foreign body reaction [16]. One of the consequences of this interaction within the host is that the PP material shrinks [7]. Recent studies also have shown that PP meshes are not inert and their pore sizes may reduce in size but also expand when they are exposed to different basic laboratory chemicals [4]. In the same study, a wide range of alterations in pore size, from −40% to 58.5%, also were seen in materials explanted after infection, recurrences, or another surgery. It is important to remember that an increase in pore size is generally equivalent to material shrinkage [17].

Most of the experimental studies with PP meshes have shown a variable grade of shrinkage after different periods of time (Table 5) [18–21], although in some of them this shrinkage was imperceptible [22,23]. Nevertheless, these studies are somewhat heterogeneous because several variables can affect the different outcomes: creation of a muscle

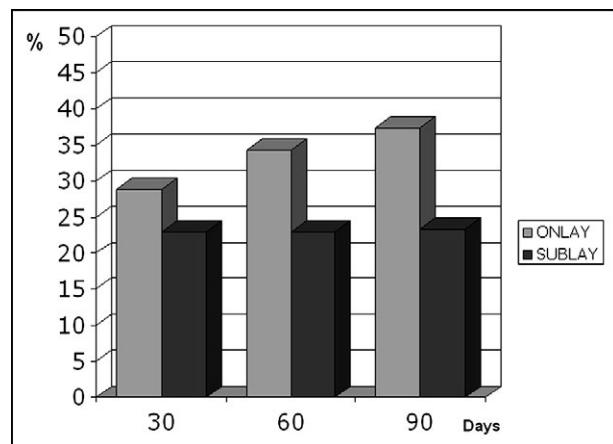


Fig. 2. Percentage of mesh shrinkage for both groups in the 3 periods of death. □, Onlay; ■, sublay.

Table 4

Cellularity (cells/mm<sup>2</sup>) at 30, 60, and 90 days after implantation

|                                                         | Day 30     |             | Day 60     |             | Day 90     |             |
|---------------------------------------------------------|------------|-------------|------------|-------------|------------|-------------|
|                                                         | Mesh onlay | Mesh sublay | Mesh onlay | Mesh sublay | Mesh onlay | Mesh sublay |
| Mononuclear cells (macrophages, histiocytes, monocytes) | 45 (9)     | 33 (7)      | 39 (8)     | 29 (9)      | 26 (7)     | 21 (3)      |
| Fibroblasts, myofibroblasts                             | 12 (2)     | 8 (1)       | 15 (4)     | 11 (3)      | 15 (3)     | 10 (2)      |
| Vascular cells                                          | 37 (6)     | 18 (4)      | 34 (10)    | 16 (4)      | 33 (6)     | 14 (1)      |
| Neutrophils                                             | 12 (2)     | 6 (3)       | 7 (1)      | 7 (2)       | 8 (2)      | 5 (1)       |
| Giant cells                                             | 8 (2)      | 6 (1)       | 8 (1)      | 7 (0)       | 6 (0)      | 5 (1)       |

Data are expressed as mean (SD).

and fascial defect, the physiologic growth of the animal, fixation of the mesh, pore size of the mesh, textile structure, weave configuration, fiber diameter, and the quantity of the material.

It is well known that inflammatory reactions also vary between different polypropylene meshes [24] and also between individuals [6]. In a study comparing shrinking of PP meshes with or without fixation after 90 days, the fixation group shrank less and retained their original shapes [18]. Reduction in the amount of implanted PP also generates less inflammatory response [25,26], and larger pore size seems to improve the mature collagen deposition between the fibrils [27]. In a recent study fewer multinucleate giant cells and foci of inflammatory leukocytic exudates were seen in multifilament PP implanted between the *erectus spinae* muscles in rats [28], although in another study monofilament high-weight PP in an intra-abdominal position showed larger granuloma formation than multifilament PP, but fewer reactions were observed in the monofilament low-weight PP [29]. Our microscopic results showed a slightly more inflammatory response of leukocytes and mononuclear cells in the onlay group. It is possible that the wound healing in the onlay position is subjected to more tensile forces or that the inflammatory response to the mesh also may depend on the interface of surrounding tissues. Another issue that could be assessed in our study was the possible influence on the acute inflammatory response of the mesh of one side to the other. In an experimental study of mesh implantation on both sides of the abdominal wall with PP and polyester, no difference was observed in mesh contrac-

tion when a PP mesh was placed near another PP mesh or next to a polyester mesh [21].

In the clinical setting the tendency to shrink also has been described. Amid [7] observed a 20% theoretic shrinkage in a radiographic follow-up evaluation after PP mesh implantation. This contraction of PP materials is particularly remarkable after the use of 3-dimensional meshes. The volume of these plugs has been described to be reduced as much as 70% and may be responsible for some complications attributed to these meshes such as recurrence, migration, and infection [8,30,31]. Mesh contraction also has been observed after the use of other types of materials such as expanded polytetrafluoroethylene, polyester, and polyethylene terephthalate [20–23]. In an interesting clinical study the intraperitoneal mesh placement of expanded polytetrafluoroethylene considerably reduced the size of the rectus abdominis fascia defect [32]. These investigators attributed this phenomenon to the fibrous ingrowth on the rough surface of the mesh acting as a scaffold for contractile forces of the muscles.

We completely agree with LeBlanc [8] that mesh shrinkage is not a complication of the biomaterial but a consequence of the incorporation of the mesh to a scar tissue that shrinks as it matures. Wound healing is an extraordinarily complex process. At the end of the inflammatory phase, about 4 days under normal conditions, the macrophages provide the growth factors necessary to stimulate the recruitment of fibroblasts, which will play the main role in the incoming fibroplastic phase [33]. This phase is in part mediated by T lymphocytes [34]. During the second week of

Table 5

Published reports that have considered PP mesh shrinkage

| Study                           | PP mesh                | Place of implantation  | Creation of defect | Animal (number of animals) | Days until death | % area reduction |
|---------------------------------|------------------------|------------------------|--------------------|----------------------------|------------------|------------------|
| Klinge et al [19], 1998         | Marlex <sup>a</sup>    | Sublay (preperitoneal) | No                 | Dogs (10)                  | 180              | 34%              |
| Zieren et al [18], 1999         | Prolene <sup>b</sup>   | Sublay                 | Yes                | Rats (30)                  | 90               | 17%–22%          |
| Zieren et al [22], 2002         | Prolene <sup>b</sup>   | Sublay                 | Yes                | Pigs* (12)                 | 100              | .01%             |
| Gonzalez and Ramshaw [23], 2003 | Marlex <sup>a</sup>    | Sublay (preperitoneal) | No                 | Pigs (6)                   | 96               | .02%             |
| Johnson et al [20], 2004        | Sepramesh <sup>c</sup> | Sublay (preperitoneal) | No                 | Rabbits (12)               | 150              | 32.6%            |
| Gonzalez et al [21], 2005       | Surgipro <sup>d</sup>  | Sublay                 | No                 | Pigs                       | 90               | 15%–65%          |

\* Fast-growing animals.

<sup>a</sup> Manufactured by Bard Inc., Murray Hill, NJ.<sup>b</sup> Manufactured by Ethicon, Somerville, NJ.<sup>c</sup> Manufactured by Genzyme, Cambridge, MA.<sup>d</sup> Manufactured by Autosuture-Tyco, Norwalk, CT.

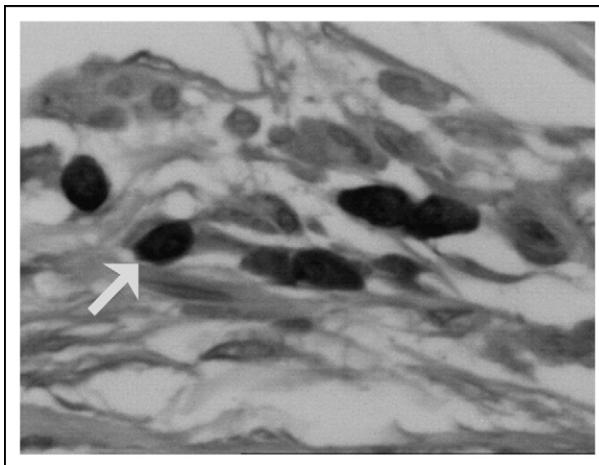


Fig. 3. Histologic appearance obtained at 30 days after implantation. Some myofibroblasts stained with desmin can be seen in the tissue surrounding mesh fibers (arrow).

healing and having started the collagen deposition, some fibroblasts assume a myofibroblast phenotype with large bundles of actin microfilaments (Fig. 3) [35]. The activity of these myofibroblasts is responsible for wound contraction and is stimulated by several growth factors, integrin receptors, and cross-links between collagen filaments. Shrinking of the mesh embedded in wound healing therefore may be attributed to myofibroblasts [17]. In an experimental model of mesh implantation in rats, spindle-shaped fibroblasts increased from months 2 to 4 and stabilized thereafter [36].

We conclude that PP meshes undergo an important degree of shrinkage that occurs during the scarring and remodeling process. In this experimental model this shrinkage has been smaller when the biomaterials were implanted in the sublay retromuscular position than when they were placed using an extrafascial onlay technique.

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